

Growth and phosphorus uptake of potato (*Solanum tuberosum* L.) in an alkaline soil as affected by mineral nitrogen forms and inoculation with phosphate-solubilizing bacteria and mycorrhizal fungi.

Kristina Weimers



Growth and phosphorus uptake of potato (*Solanum tuberosum* L.) in an alkaline soil as affected by mineral nitrogen forms and inoculation with phosphate-solubilizing bacteria and mycorrhizal fungi.

Hur tillväxt och fosforupptag hos potatis (*Solanum tuberosum* L.) odlad i en jord med högt pH påverkas av mineralkväveform och inokulering med fosforlösande bakterier och mykorrhiza.

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Preface

This bachelor's thesis is the result of a degree project at level C at the Master of Science programme in Horticulture, performed at the Department of Biosystems and Technology at SLU, Alnarp. I would like to take the opportunity to thank Anders Andersson for the gift of soil from his farm and Dr. Venkatesh Devanur at AgriLife in India for his generous gift of *Bacillus megaterium*, strain MCC 0053, provided as a soil inoculum. I would also like to thank Siri Caspersen for guidance and supervision during the project, Jan-Eric Englund for help with interpretation of the statistical results, Göran Nilsson at the Biotron for help during the cultivation in the climate chambers, David Ivarsson and Lucy Clark from Trädgårdslabbet for invaluable help with irrigation during some critical summer weeks and Helene Larsson Jönsson for advice and generous lending of equipment.

Abstract

In soils with relatively high pH ($\text{pH} > 7$), potatoes (*Solanum tuberosum* L.) and other phosphorus (P) demanding crops might suffer from P deficiency despite P fertilization and significant reserves of P in the soil. Also, a high risk for P deficiency for potatoes may be expected when the soil is cold and the root system undeveloped. Therefore, the risk of P-limitation in high-pH soils is probably greatest for early varieties. In this study a trial was set up with two objectives: (i) To assess the effect of ammonium sulphate (T1) on P uptake, vegetative growth and tuber yield in potatoes grown without addition of P in soils with relatively high pH (7.5) and P-AL class IVB (12.5 mg P/100 g soil), under climatic conditions similar to those for early potatoes in the south of Sweden. The effect of ammonium sulphate was compared with the effect of ammonium nitrate (T2), ammonium chloride (T3) and potassium nitrate (T4). (ii) To assess the impact of inoculation with spores of arbuscular mycorrhizal fungi (AMF) *Rhizophagus irregularis* (T5) and the phosphorus solubilizing bacteria (PSB) *Bacillus megaterium* (T6) on the said parameters and under the same conditions. The trial was limited to the early potato variety 'Solist' grown with one level of nitrogen fertilizer. After harvesting, data was collected on fresh and dry matter and shoots and tubers were analysed for P content. The potato plants in the trial did not reach full maturity, probably due to salt toxicity, which made any possible differences between the treatments difficult to discern. At harvest the P concentration in all plants in all treatments was lower than normal levels. No significant differences were found between treatments on the effect on total dry weight of tubers or shoots. Also, no significant differences were found between treatments in number of tubers produced. The plants which received ammonium chloride (T3) had a significant smaller concentration of P compared to T1, T2 and T5. The difference can probably be explained by the fact that T3 had the highest salt concentration of all treatments. AMF colonization in the roots were found in all pots in both the control T2 and the inoculated T5. In conclusion, the result did not support the hypothesis that it would be possible to control P availability for early potatoes, grown in alkaline soils with relatively high P content, through the choice of nitrogen source or through inoculation with PSB or AMF.

Table of Contents

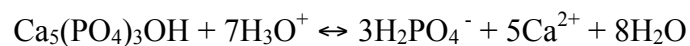
| | |
|-------------------------------------------------------------------------------------|----|
| Introduction | 6 |
| <i>P</i> -minerals | 6 |
| Adsorbed <i>P</i> | 6 |
| Organic <i>P</i> | 7 |
| <i>P</i> _i in soil solution | 7 |
| Optimal pH for <i>P</i> plant uptake | 7 |
| The effect of NH ₄ and NO ₃ on plant <i>P</i> nutrition | 8 |
| <i>Plant vigor</i> | 8 |
| Potato and <i>P</i> | 9 |
| <i>P</i> in agricultural soil | 9 |
| Microbial Inoculants | 9 |
| <i>Mycorrhiza inoculation</i> | 9 |
| <i>PSB</i> | 10 |
| Aims and objectives | 13 |
| Material and Methods | 14 |
| Material | 14 |
| <i>Plant material</i> | 14 |
| <i>Soil and soil nutrition status</i> | 14 |
| <i>Inoculum</i> | 14 |
| Methods | 15 |
| <i>Experimental design</i> | 15 |
| <i>Nutrient requirements</i> | 15 |
| <i>Treatments</i> | 16 |
| <i>Planting</i> | 18 |
| <i>Site and climate</i> | 19 |
| <i>Irrigation</i> | 20 |
| <i>Data collection</i> | 20 |
| <i>Statistics</i> | 21 |
| Results | 22 |
| Visual observations | 22 |
| Vegetative weight | 22 |
| Number of tubers | 23 |
| Water consumption | 23 |
| <i>P</i> content at harvest | 24 |
| Mycorrhizal colonisation | 26 |
| Discussion | 27 |
| <i>P</i> deficiency related to soil test results | 27 |
| <i>P</i> deficiency related to pH | 29 |
| The effect of ammonium sulphate versus ammonium chloride | 29 |
| The effect of <i>Bacillus megaterium</i> and mycorrhizal fungi | 32 |
| Conclusions | 33 |
| References | 35 |

Introduction

P-minerals

P exists in different forms in soil. A first distinction can be made between inorganic P and organic P. The biggest inorganic fraction is the primary minerals, consisting essentially of apatite, $\text{Ca}_{10}(\text{PO}_4)_6\text{X}_2$ where X can be F^- , Cl^- or OH^- , where P is tightly bound to calcium (Ca) (Havlin *et al.*, 2005). The slow weathering of apatite is the main origin of the naturally occurring P in our soils (Eriksson, 2011). Over time the phosphate ions (Pi) released by weathering has been assimilated by plants and microorganisms, or has been chemically bound to iron (Fe), aluminium (Al) or Ca and formed secondary minerals. As the P bound in old minerals is hard to access due to the well crystallized and stable compounds, the P in the younger, more recently formed minerals is more readily available (Sims and Sharpley, 2007).

A precipitation - dissolution equilibrium governs the solubility of P minerals (Hinsinger, 2001). This equilibrium is under direct dependence of pH and the concentration of P ions and metal ions such as Ca, Al or Fe and in the soil solution. This is shown by the following equation illustrating the weathering of hydroxyapatite:



According to Hinsinger (2001), the mobility of P can, however, probably only be predicted on the basis of such precipitation - dissolution equilibria in the most alkaline pH range ($\text{pH} > 8$). In less alkaline soils, which comprises most agricultural soils, other processes, such as adsorption - desorption reactions, are involved in the observed relationship between the mobility of soil P and pH.

Adsorbed P

Another fraction of inorganic P is the Pi adsorbed to soil minerals and P-sorbing surfaces, such as metal oxides, clay minerals and organic matter (Havlin *et al.*, 2005). As described by Hinsinger (2011), metal oxides, primarily Fe and Al oxides, have a strong reactivity as sorbents and therefore play an important role in most soils in the adsorption of P. As the positive charge of these minerals increase with decreasing pH, so do their ability to adsorb anions such as P. The concentration of Pi and competing anions in the soil determine the

adsorption - desorption equilibrium, as desorption of sorbed P takes place mainly via anion and ligand exchange reactions (Hinsinger, 2001).

Organic P

The organic P is the P bound in the living biomass and dead organic matter in the soil. Organic P constitutes on average 40 - 50 % of total P in Swedish agricultural soils (Gunnarsson, 1987), but the proportion of organically bound P can vary greatly, mainly depending on the humus content. A large proportion of the organic P in soil is still poorly characterized, but a major component is usually inositol phosphates (Havlin *et al.*, 2005). The organic P becomes available to plants through the action of phosphatase enzymes which are produced by 70 - 80 % of the microbial population (Sylvia, 1998).

P_i in soil solution

The forms in which P_i occur in the soil solution changes according to pH. The pH has a direct effect on the speciation of P through the dissociation of orthophosphoric acid (H₃PO₄) to the plant-available orthophosphate ions H₂PO₄⁻ and HPO₄²⁻ (Marschner, 1995). Between pH 2.1 and 7.2 most P_i are present in the form of H₂PO₄⁻ and the major species at pH above 7.2 is HPO₄²⁻ (Lindsay, 1979).

As described by Hinsinger (2001), H₂PO₄⁻ and HPO₄²⁻ have a strong tendency to form ion-ion pairs or complexes with several metal ions such as Fe, Al, Ca or Mg. Which ion-ion pairs or complexes that are formed depend on the soil pH as the occurrence of metal ions is primarily determined by pH. The solubility of Fe and Al oxides is increased in acid soils, resulting in high concentrations of Fe and Al-ions in the soil solution. In neutral or alkaline soils Ca, and to some extent Mg, are the dominating cations. The presence of competing ligands, such as the organic ligands citrate and oxalate, will influence to what extent the P ions will bind to the metal ions (Hinsinger, 2001).

Optimal pH for P plant uptake

As described above, the solubility and bioavailability of P is strongly tied to soil pH as pH determines the solubility and concentration of Fe, Al, Ca or Mg in the soil solution (Hinsinger, 2001). Besides forming dissolved ion-ion pairs, the metal ions precipitates with P ions, forming iron and aluminium phosphates in acid soils, and calcium phosphates in alkaline

soils. The maximum solubility of P occurs around pH 6.5, where the concentration of Al and Fe ions on one hand, and Ca ions on the other hand, is minimized (Sims and Sharpley, 2007).

The effect of NH_4 and NO_3 on plant P nutrition

Plants can assimilate N in different forms, the main ones being nitrate (NO_3^-) and ammonium (NH_4^+) (Havlin *et al.*, 2005). The two ions differ in terms of plant assimilation and may have different effect on biomass accumulation. The uptake capability and use of ammonium or nitrate varies depending on several factors such as the plant species and development stage, and the chemical conditions in the rhizosphere (Fan *et al.*, 2009).

It is well documented that roots generally excrete H^+ when they take up NH_4^+ and therefore decrease pH in the rhizosphere, whereas uptake of NO_3^- leads to alkalization of the rhizosphere (Marschner, 1995). The microbial nitrification of NH_4^+ to NO_3^- also produces H^+ (Johnson *et al.*, 2010). As the solubility of inorganic P changes according to soil pH, the choice of N source may influence plant P availability. Accordingly, supplying N as NH_4^+ in alkaline soils have in several studies been shown to increase P uptake (Fan *et al.*, 2009; Marschner *et al.*, 1987; Zhang *et al.*, 2004).

Plant vigor

Besides P availability in the rhizosphere, the size and vigor of the root system influence the plants P uptake capacity (Taiz *et al.*, 2015). Ammonium and nitrate have different effects on biomass accumulation and root vigor in different plant species (Marschner, 1995). It has been assumed that only species that are usually growing in acid soils or in cold climates, where NH_4^+ is the dominating N source, have a preference for NH_4^+ (Gigon and Rorison, 1972; Marschner, 1995). Potato should, according to this reasoning, prefer NO_3^- . But there is evidence that crops may be more productive when supplied with both NO_3^- and NH_4^+ (Cao and Tibbitts, 1993), and mineral fertilizers recommended for potatoes in Sweden contain both forms in approximately equal proportions. A commonly used fertilizer for potatoes, YaraMila PROMANGA 11-5-18, contains 6.6 % $\text{NH}_4\text{-N}$ and 4.4 % $\text{NO}_3\text{-N}$ (Yara, 2016b).

Potato and P

Plants need a steady supply of phosphorus (P) for normal development. P is involved in several important processes in the plant such as the construction of carbohydrates, cell membranes, DNA, RNA and the energy transporting ATP-molecule (Marschner, 1995). Therefore, lack of P generally results in reduced speed of important processes such as cell division, cell expansion, photosynthesis and respiration, with reduced growth as a result.

The risk for deficiency is highest in calcareous soils at temperatures below 10 °C (Bennett, 1993). Thus the problem is greatest early in the season when the soil is cold and the root system undeveloped.

P in agricultural soil

There are significant reserves of P in Swedish agricultural soils. A study conducted in Skåne in the 1980's found a total amount of 1500 - 2500 kg P per hectare (Gunnarsson, 1987). A normal potato harvest carry away 10 - 30 kg P per hectare (Yara, 2016a). Despite this, providing enough P to potatoes and other P demanding crops can be difficult, especially in alkaline and calcareous soils because of the formation of poorly soluble calcium phosphate minerals (Havlin *et al.*, 2005).

Microbial inoculants

Mycorrhiza inoculation

Arbuscular mycorrhizal fungi (AMF) are soil fungi that has developed a symbiotic relationship with plants over the last 455 million years (Redecker *et al.*, 2000). The majority of agricultural plants, including potato, form symbiosis with AMF (Hijri, 2016). The mycelium of the fungi act as an extender of the root system and benefits for the host plant include enhanced water relations, better resistance to pathogens and improved supply of nutrients of low mobility in the soil solution, particularly P (Smith and Read, 2008). In low P soils the infection by AMF in plant roots might result in increased growth compared to non-infected plants (Smith and Read, 2008). If the P content of the soil is high, the plant might limit the AMF colonization of their roots, probably as a way to limit the carbon cost of the symbiosis (Hayman, 1983).

Black and Tinker (1977) reported the first successful field inoculation experiment on potato, and since then numerous studies have been conducted on the effects of AMF inoculation on potato production (Douds *et al.*, 2007; Duffy and Cassells, 2000; McArthur and Knowles, 1993; Vosátka and Gryndler, 2000; Yao *et al.*, 2002). The results depend on inoculant used, potato cultivar and P status of the soil, but overall the results of the studies have shown that inoculation with AMF allows higher yields and larger tubers compared with non-inoculated controls treatments using conventional chemical fertilizers (Douds *et al.*, 2007; Wu *et al.*, 2013).

In 2016, Hijri reported an analysis of the largest dataset available so far for AMF inoculation under authentic field conditions. The dataset consisted of 231 field trials in North America and Europe where the same AMF inoculant (*Rhizophagus irregularis* DAOM 197198) was applied to potato during a four-year period. The analysis showed that the average yield increase for the inoculated fields was 3.9 tons/ha (9.5 % of total crop yield). The inoculation was profitable already at a yield increase of 0.67 tons/ha. Almost 79 % of all trials had a yield increase above that level.

Numerous commercial mycorrhizal-based inoculants are available worldwide. They are either produced *in vivo* using pot co-culture methods or *in vitro* in bioreactors (Hijri, 2016).

PSB

A range of phosphate solubilizing bacteria (PSB) participate in the solubilisation of calcium bound P in the rhizosphere (Marschner, 2012). Phosphate solubilizing bacteria contribute to the solubilisation mainly by excreting organic acids, such as gluconic, oxalic and citric acids (Richardson, 2001). The organic acids bring P into soil solution by directly dissolving rock phosphate or by chelating calcium ions (Kucey, 1983). Phosphate solubilizing bacteria are also involved in the mineralisation of organic P through the production of phosphatase enzymes (Sylvia, 1998).

It has been suggested that the P solubilisation by microorganisms might be a major mechanism for plant growth promotion (Richardson, 2001). However, the ability to solubilize P alone does not indicate that a bacterium has plant growth promoting properties (Vessey, 2003). Biotic factors such as synergy effects and antagonism between competing

microorganisms, and abiotic environmental factors, have a severe influence on the plant growth promoting properties of a certain bacterium (Malboobi *et al.*, 2009).

PSB as a biofertilizer in potato

As a way to find inexpensive and environmentally benign alternatives to mineral fertilizers, attention has been drawn to PSB as biofertilizers (Zaidi, 2015). Several studies conducted on potato have shown positive results (Ekin *et al.*, 2009; Faccini *et al.*, 2007; Hosni *et al.*, 2016; Malboobi *et al.*, 2009; Naderi *et al.*, 2012).

An Iranian experiment conducted by Malboobi *et al.* (2002) studied the synergism between three PSB isolates when used as inoculum in potato: the acid producing bacteria *Pantoea agglomerans* and *Microbacterium laevaniformans*, and the strong phosphatase producer *Pseudomonas putida*. The bacteria were applied to potato grown in laboratory experiments, greenhouse trials and field soils. Consistently, the mixture of *P. agglomerans* or *M. laevaniformans* and *P. putida* led to higher biomass and enhanced tuber growth both in greenhouse and field trials. Double inoculation of the acid producing *P. agglomerans* and *M. laevaniformans* resulted in a yield increase of 20 - 25 %. The authors concluded that double inoculation of the described bacteria can be recommended as biofertilizers for potato (Malboobi *et al.*, 2009).

In a field trial in the calcareous soil of Isfahan in Iran, conducted by Naderi *et al.* (2012), two different methods of PSB application on potato were studied together with different levels of chemical phosphate fertilizer. A PSB biofertilizer named Barvar2 made by “Green Biotech Co. Iran” was used, with the bacteria not specified. The result showed that inoculation with PSB had a significant effect on tuber yield and tuber mean weight. The best results were obtained when PSB were sprayed on the soil and 100 kg/ha phosphate fertilizer was applied (Naderi *et al.*, 2012).

A third Iranian study conducted by Hosni *et al.* (2016), showed that inoculation of potato minitubers with *Pseudomonas* spp. and two species of *Bacillus* (*B. megaterium* and *B. subtilis*) had a significant positive effect on tuberization and yield, both as dual and separate inoculation (Hosni *et al.*, 2016).

In a field experiment in the Andes of Colombia, Faccini *et al.* (2007) found that inoculation with the PSB *Pseudomonas cepacia*, *Xanthomonas maltophilia*, *Enterobacter cloacae* and *Acidovorans delafieldii* allowed equivalent potato yields with half the P fertilizer when applied together with four strains of the asymbiotic nitrogen fixer *A. chroococcum* (Faccini *et al.*, 2007).

Inoculation of potato with *Bacillus* sp. was tested by Ekin *et al.* under field conditions in Turkey 2006 and 2007 at different levels of nitrogen fertilization. The result showed that yields were higher at all levels of nitrogen fertilization in the inoculated plots compared to the control in both years (Ekin *et al.*, 2009).

Aims and objectives

In this study, a trial was set up to assess the possibility to control P availability for early potatoes grown in soils with relatively high pH (7.5) and P-AL class IV (12.5 mg P/100 g soil), without addition of P and under climatic conditions similar to those for early potatoes in the south of Sweden. The study was designed with two objectives:

- (i) To assess the possibility to control P availability by the choice of nitrogen source, the impact of ammonium sulphate on P uptake, vegetative growth and tuber yield in early potatoes was studied. The effect of ammonium sulphate was compared with the effect of ammonium nitrate, ammonium chloride and potassium nitrate. Ammonium sulphate is known to have a greater acidifying effect than the other N-fertilizers used in the trial (Khonje *et al.*, 1989). Therefore, the hypothesis was that this treatment would result in the highest plant P uptake.
- (ii) To assess the impact of inoculation with the phosphorus solubilizing bacteria (PSB) *Bacillus megaterium* and spores of arbuscular mycorrhizal fungi (AMF) *Rhizophagus irregularis* on P uptake, vegetative growth and tuber yield in early potatoes. As both *Bacillus* spp. and *Rhizophagus irregularis* has shown to have a positive impact on P uptake in potatoes (Ekin *et al.*, 2009; Hosni *et al.*, 2016, Hijri, 2016) the hypothesis tested was that plants in these treatments would have a higher P uptake than the control.

The trial was limited to the early potato variety ‘Solist’ grown with one level of nitrogen fertilization. The inocula of *Bacillus megaterium* and *Rhizophagus irregularis* were studied one concentration each together with ammonium nitrate.

Material and Methods

Material

Plant material

The early potatoes cultivar 'Solist' (*Solanum tuberosum* L.), was chosen for the trial. This cultivar produces around 30 kg tubers per hectare if allowed to reach full maturity (Hagman, 2011). The sowing material was produced by LPM-potatis in Laholm and bought from the retailer Blomsterlandet on April 6th. The tubers already had small white sprouts (0.1 - 1.5 cm) at delivery. The potatoes were placed on plastic trays for further sprouting for three weeks in a heated greenhouse ventilated at 18 °C and with additional lightning between 7 a.m. and 11 p.m. The tubers were sprayed with water every second day to avoid excessive water loss. At planting on April 27th the tubers weighed 90 - 158 grams and had 1 - 3 cm chubby green sprouts.

Soil and soil nutrition status

Soil was collected from a field on the potato farm Hörtegården in Skivarp in the south of Skåne in Sweden (55°23'50.3"N 13°32'40.0"E) in the middle of April. A soil analysis gave the following result: pH 7.5, P-AL 12.5 mg/100 g (P-AL class IVB), P Olsen 21 mg/1000 g, K-AL 8.5 mg/100 g (K-AL class III), Mg-AL 12 mg/100 g, Ca-AL 330 mg/100 g, and K/Mg 0.7. The humus content was 3.1 % and the clay content 17 %.

Inoculum

Mycorrhiza inoculum

Rhizophagus irregularis spores produced under sterile conditions using an *in vitro* root-organ culture production system, mixed with fine calcined attapulgit at a concentration of 10 000 spores/gram, was provided by the German company Symplanta GmbH & Co. The mycorrhiza carrier, pure calcined attapulgit, was purchased from the same company (Symplanta GmbH & Co, 2016).

Bacteria inoculum

Bacillus megaterium of the strain MCC 0053 was a gift from the company AgriLife in India. It is sold in an inoculum containing endospores of the bacteria under the product name P SOL

B (Agrilife, 2016). The concentration was 1.4×10^9 CFU/gram and the carrier material was talc (unspecified formula).

Methods

Experimental design

The experiment was set up as a completely randomised design consisting of six treatments with six replicate pots per treatment. In total, 36 potato tubers were planted. The containers were randomly placed in a climate chamber and moved twice a week.

Nutrient requirements

The macronutrient requirements of ‘Solist’ were calculated using the recommendations for new potatoes from the Swedish Board of Agriculture (Albertsson *et al.*, 2015). An estimated yield of 30 tonnes per hectare and an 84 days growing season were used for the calculations (Albertsson *et al.*, 2015; Hagman, 2011). The amount of potassium was adjusted according to the recommendations to avoid a too low K/Mg ratio. The amounts of micronutrients were calculated on the basis of the content in a common commercial fertilizer used by potato farmers, YaraMila Pro Magna 11-5-18 (table 1) (Yara, 2016b). No phosphorous was added.

Table 1: The nutritional requirements of ‘Solist’ with a yield of 30 tonnes/hectare and an 84 days growing season (Albertsson *et al.*, 2015, Yara, 2016a, Yara 2016b).

| Nutrient | kg/ha |
|----------|-------|
| N | 100 |
| K | 210 |
| Mg | 10 |
| Fe | 0.3 |
| Mn | 2.27 |
| Zn | 0.36 |
| Cu | 0.27 |
| Mo | 0.02 |
| B | 0.45 |

To calculate the nutrient requirements for each pot, the share of a hectare that each potato plant has access to when grown in field was used. The smallest plant spacing recommendations, 0.25 m between the plants and 0.55 m between the rows, were used for the calculation. This gave each plant 0.137 m^2 , which was 2.2 times the area of the pot’s soil

surface. The number was rounded down to twice the soil surface's area, giving 0.126 m² to each plant (or 0.126 * 10⁻⁵ hectare).

Treatments

Table 2 and 3 lists the substances added to each pot. Table 4 lists the nutrient content in the substances applied to the pots. The amount of S and Cl varied between the treatments: T1 contained 1527 mg S per pot (242 kg/hectare), T2, T3, T5 and T6 contained 805 mg S (38 kg/hectare) and T4 contained 83 mg S (13.2 kg/hectare). T3 was the only one containing chloride: 1595 mg/pot which corresponds to 253 kg/hectare.

Table 2: The treatments and the variables tested.

| Treatment | | mmol/pot | Variable tested |
|-----------|-------------------------------------------------------|------------|------------------------------------------------------------|
| T1 | (NH ₄) ₂ SO ₄ | 45 | (NH ₄) ₂ SO ₄ |
| | K ₂ SO ₄ | 34 | |
| T2 | NH ₄ NO ₃ | 45 | NH ₄ without the acidifying effect of T1 |
| | K ₂ SO ₄ | 34 | |
| T3 | NH ₄ Cl | 90 | Control SO ₄ in T1 |
| | K ₂ SO ₄ | 34 | |
| T4 | KNO ₃ | 68 | Control NO ₃ in T2 |
| | Ca(NO ₃) ₂ x 4H ₂ O | 11 | |
| T5 | NH ₄ NO ₃ | 45 | The effect of <i>Rhizofagus irregularis</i> compared to T2 |
| | K ₂ SO ₄ | 34 | |
| | Mycorrhiza inoculum ¹ | 2.40 grams | |
| T6 | NH ₄ NO ₃ | 56 | The effect of <i>Bacillus megaterium</i> compared to T2 |
| | K ₂ SO ₄ | 34 | |
| | <i>Bacillus megaterium</i> ² | 0.20 grams | |

1) *Rhizofagus irregularis*, inoculum produced by Symplanta GmbH & Co, 10 000 spores/gram.

2) Product name P SOL B, produced by AgriLife, www.agrilife.in, 1.0 * 10⁹ CFU/gram.

Table 3: Substances added to all pots

| | | mmol/pot |
|----------------|-------------------------------------------------------------------------------------------------------|-------------|
| Macronutrients | MgSO ₄ x 7H ₂ O | 5.2 |
| | CaSO ₄ x 2H ₂ O ¹ | 11 |
| Micronutrients | Fe-EDTA 5 % Fe | 0.082 |
| | MnSO ₄ x 5H ₂ O | 0.260 |
| | ZnSO ₄ x 7H ₂ O | 0.035 |
| | CuSO ₄ x 5H ₂ O | 0.027 |
| | Na ₂ MoO ₄ x 2H ₂ O | 0.001 |
| | H ₃ BO ₃ | 0.295 |
| AMF carrier | Calcined attapulgite (Mg,Al) ₂ Si ₄ O ₁₀ (OH) x 4H ₂ O | 16.16 grams |

1) 0 mmol was added to the pots in T4.

Table 4: Total amount of fertilizer applied to each pot.

| Nutrient | Treatment | kg/ha | mg/pot |
|----------|---------------|-------|--------|
| N | All | 100 | 630 |
| K | All | 210 | 1323 |
| Mg | All | 10 | 63 |
| S | 1 | 242 | 1527 |
| | 4 | 13 | 83 |
| | 2, 3, 5, 6 | 38 | 805 |
| Ca | All | 35 | 220 |
| Fe | All | 0.73 | 4.6 |
| Mn | All | 2.27 | 14.3 |
| Zn | All | 0.36 | 2.3 |
| Cu | All | 0.27 | 1.7 |
| Mo | All | 0.02 | 0.1 |
| B | All | 0.45 | 2.9 |
| Cl | 3 | 253 | 1595 |
| | 1, 2, 4, 5, 6 | 0 | 0 |

Preparation of nutrient solutions

The micronutrient solution was mixed in one batch to serve all pots and the macronutrient solutions were mixed separately for each treatment. The solutions were diluted to a volume where 0.5 L of both the micronutrient and macronutrient solution were given to each plant at the two fertilization occasions.

Mycorrhiza inoculum

The soil in each pot in T6 was mixed with 2.4 g inoculum (24 000 spores), resulting in an initial concentration of 1.92 spores/gram dry soil. The number of spores were calculated on basis of the number of AM-diaspores observed in a 28 years long Swedish study on arable land where no phosphorus fertilizer was applied (Mårtensson and Carlgren, 1994).

Bacteria inoculum

To each pot in T7, 200 mg of the bacteria inoculum was mixed into the bottom 10 centimetres of soil ($2.8 * 10^8$ CFU/pot, $4.48 * 10^4$ CFU/gram dry soil in the bottom 10 centimetres). The amount of inoculum added was calculated using Argillite's recommendations for field application, and doubled to compensate for the low organic content in the soil, as AgriLife recommended to mix the inoculum with compost before applying it to the field.

Fertilisation strategy

Fifty percent of the macronutrient solution was given at planting, and fifty percent one month after the sprouts had emerged, on June 11th. All of the micronutrient solution was given at planting.

Planting

Planting took place on April 26th. Before planting, the soil was sifted through a 1 cm mesh and homogenised by shovelling. Thirtysix pots of 12.5 L, measuring 28 cm in diameter at the top, were filled with 12.50 kg soil (dry weight at 105 °C), corresponding to a volume of approximately 11.5 L. The pots were divided into 6 groups of 6 pots, representing 6 treatments with 6 replications.

The nutrient solution was poured into the soil of each pot and the CaSO₄, mycorrhiza inoculum and mycorrhiza inoculum carrier were added dry. Before planting, the soil from each pot was mixed thoroughly. The *Bacillus megaterium* inoculum was mixed into the soil under the potato tuber in the pots of treatment 6 at planting. The carrier material of this inoculum was not added to the pots in the other treatments.

The tubers were divided into 6 sets of 6 tubers. They were chosen to form as similar sets as possible with regards to number of sprouts on the individual potatoes and total weight of the six potatoes (740 to 748 grams). The tubers were planted at 10 cm depth, 10 cm above the bottom of the pot.

Site and climate

The trial was conducted under controlled environment in a daylight climate chamber in the phytotron at the the Swedish University of Agricultural Sciences in Alnarp, during 76 days from April 27th to July 11th 2016. The humidity in the climate chamber was 70 %. The temperature was adjusted to mimic planting outside in the beginning of April in the south of Sweden, in areas where early potatoes are produced. Temperature data from Barkåkra and Sturup weather stations were analysed to find average weekly night and day temperature. The result was adjusted in order to accumulate a sufficient number of growing degree days (GDD) for ‘Solist’ to reach maturity, and to fit the conditions and restrictions of the climate chambers. This meant raising both night and day temperature during the whole trial period compared to mean temperatures found in the analysis (table 5). At harvest, 710 GDD (base 5°C) were accumulated. According to Jannie Hagman (personal communication, June 9th, 2016) ‘Solist’ reached maturity for a first harvest after 64 days in a field trial on the farm Hillarp outside Torekov in southern Sweden during the warm spring 2011. Between planting on April 6th and harvesting on June 9th, 740 GDD had been accumulated.

Table 5: Mean night and day temperature at Sturups weather station during the cultivation period for early new potatoes (SMHI, 2016). The table also shows the temperature in the climate chamber Biotron the corresponding trial week.

| Cultivation week in field | Sturup night temperature °C 2016/2015 | Sturup day temperature °C 2016/2015 | Corresponding trial week | Biotron night temperature °C | Biotron day temperature °C |
|---------------------------|------------------------------------------|----------------------------------------|--------------------------|------------------------------|----------------------------|
| April | | | | | |
| 14 | 4.5 | 9.0 | 17 | 10 | 16 |
| 15 | | | 18 | 10 | 16 |
| 16 | 3.4 | 7.5 | | 10 | 16 |
| 17 | | | 20 | 10 | 16 |
| May | | | | | |
| 18 | 8.8 | 15.1 | 21 | 10 | 16 |
| 19 | 7.8 | 11.5 | 22 | 10 | 16 |
| 20 | | | 23 | 10 | 16 |
| 21 | 7.5 | 11.4 | 24 | 12 | 18 |
| June | | | | | |
| 22 | | | 25 | 14 | 20 |
| 23 | | | 26 | 14 | 20 |
| 24 | 10.5 | 14.1 | 27 | 14 | 20 |

Irrigation

The pots were irrigated individually by weight with distilled water every 3rd - 5th day the first 6 weeks, and every 2nd – 3rd day the last 5 weeks. All pots were kept between 50 - 70 % of the field capacity (0.3 kg water/kg soil at 100 % field capacity) and allowed to reach the lower limit before irrigated to the upper limit. The pots were placed on trays in order to avoid leaking of water and nutrients.

Data collection

The following data were collected after harvest from each pot: (1) Fresh weight of shoots and tubers, (2) number of tubers over 10 mm in diameter, (3) dry weight of shoots and tubers, (4) total P content in tubers and shoots. Additionally, data was collected from the pots in T2 and T7 on the presence or absence of mycorrhizal colonization in the roots.

Fresh weight of shoots and tubers and the number of tubers were registered at harvest. After drying the plant material at 70 °C for ten days, the dry weight was measured. In order to

produce liquid samples for analyses of P-content, the dried plant material was grinded and homogenised and then combusted in nitric acid (HNO₃) under high pressure by microwave radiation (CEM Mars5). Analysis of mineral nutrient composition of shoots and tubers was performed by Eurofins Food and Agro Sweden AB, Kristianstad.

At harvest, ¼ of the root system from all the plants in T2 and T7 was washed and preserved in alcohol. The presence or absence of mycorrhizal colonization in the roots was determined by conventional root staining and microscopy (Vierheilig *et al.*, 1998).

Statistics

One-way analysis of variance (ANOVA) together with Tukey's HSD test for differences of means, with a confidence interval set to 95 %, was used for statistical analysis of the data from the experiment. The program used was Minitab Express.

Results

Visual observations

Nineteen days after emergence (dae), necrosis occurred at the tip and margin of leaves, and as small spots on the leaf surface, on some of the plants. On 26 dae, older leaves started to turn yellow. After 36 dae, 23 of 35 plants had yellow older leaves and all plants had developed necrosis at the margin of leaves and spots on the leaf surfaces. When the symptoms were graded between 0-3, the severity of the two symptoms were shown to be significantly correlated ($P < 0.001$), but there was no significant difference between treatments regarding these symptoms. At harvest on 60 dae, the foliage of all plants was seriously affected by widespread death of tissue. None of the plants went into the generative phase.

Vegetative weight

No significant differences were found between treatments on the effect on total fresh or dry matter of tubers or shoots (see figure 1 for plant dry matter). The weight of dried shoots and dried tubers were as expected significantly correlated ($P < 0.001$). Total plant dry matter (DM) were in general found to be about 19 % of fresh matter (FM). Fresh weight of the tubers from each plant varied between 270 and 400 grams (figure 2), corresponding to a yield of about 23 tonnes per hectare (row spacing 0.6 m, plant spacing 0.25 m).

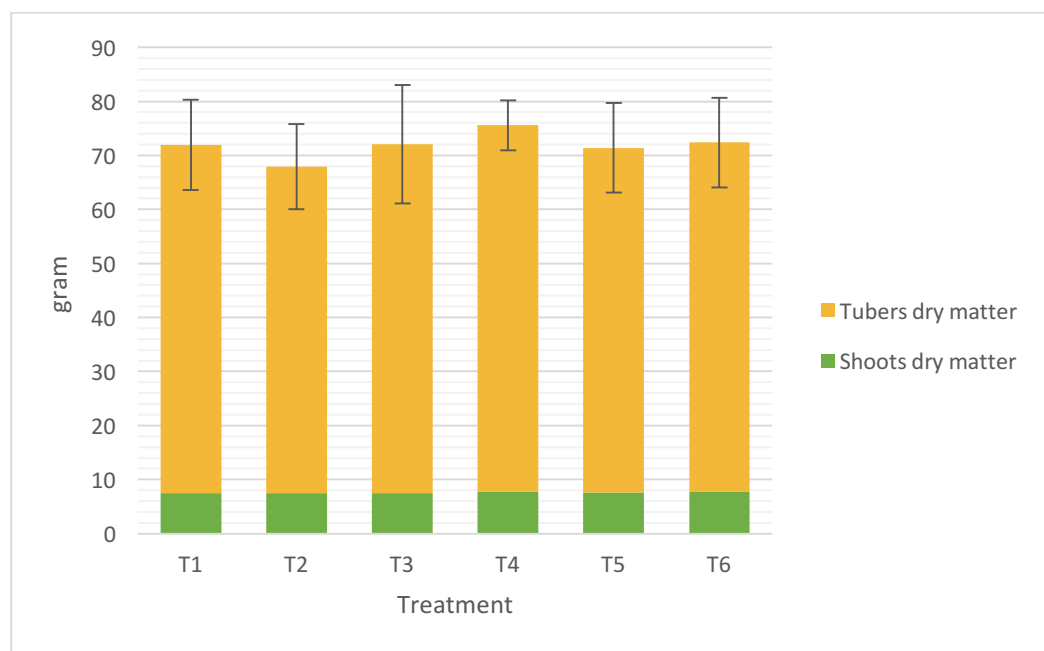


Figure 1: Plant dry matter at harvest (60 dae). Mean values, $n=6$, \pm SD.

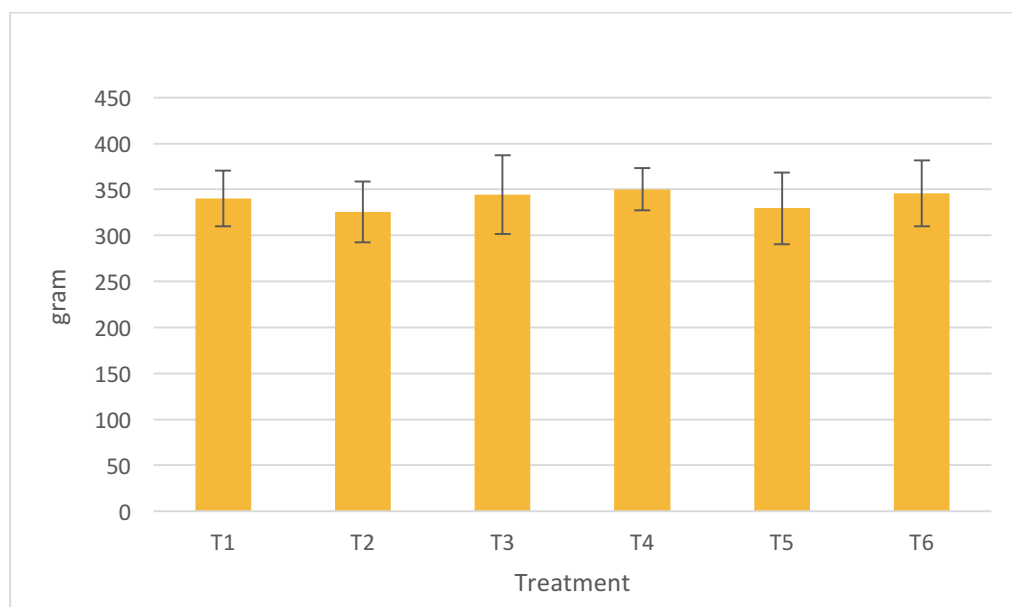


Figure 2: Fresh tuber weight per plant at harvest (60 dae). Mean values, $n=6$, \pm SD.

Number of tubers

The number of tubers from each plant varied between 9 and 21. No significant differences were found between treatments in number of tubers produced.

Water consumption

In total, the potato plants consumed around 14 L each of water from emergence of the sprouts to harvest. Tukey's HSD test for differences of means, with a confidence interval set to 95 %, did not show any significant differences in water consumption between the treatments. The Fisher LSD method, however, indicated a difference between T6 and T2, where T6 consumed significantly more water than T2 (table 6). Water consumption and total DM was significantly correlated ($p < 0.001$).

Table 6: Grouping information on total volume of consumed water using the Fisher LSD Method and 95 % confidence.

| Treatment | Mean (L) | Grouping | |
|-----------|----------|----------|---|
| T6 | 14.48 | A | |
| T4 | 14.30 | A | B |
| T3 | 13.96 | A | B |
| T5 | 13.85 | A | B |
| T1 | 13.52 | A | B |
| T2 | 13.40 | | B |

P content at harvest

The P concentration in the shoots at harvest was found to be 0.07 – 0.12 % of DM (figure 3). A One-Way ANOVA test indicated a significant difference between treatments ($p < 0.01$). The Tukey's HSD test for differences of means showed that T3 had a significant smaller concentration of P compared to T1, T2 and T5.

The P concentration in tubers varied between 0.13 % and 0.18 % (DM) (figure 3). Tukey's HSD test for differences of means did not show any significant differences between the treatments. The Fisher LSD method, however, indicated a lower P concentration in T3 compared to T1, T2 and T6.

When P content in shoots and tubers was analysed, no significant differences were found between treatments. However, T3 had the lowest mean value (fig 4). Also, when the P content in the tubers was analysed separately, no significant differences between treatments were found. However, T3 had the lowest mean value also in this case (fig 4). P content of shoots and tubers was significantly correlated with DM of shoots and tubers respectively ($p < 0.0001$).

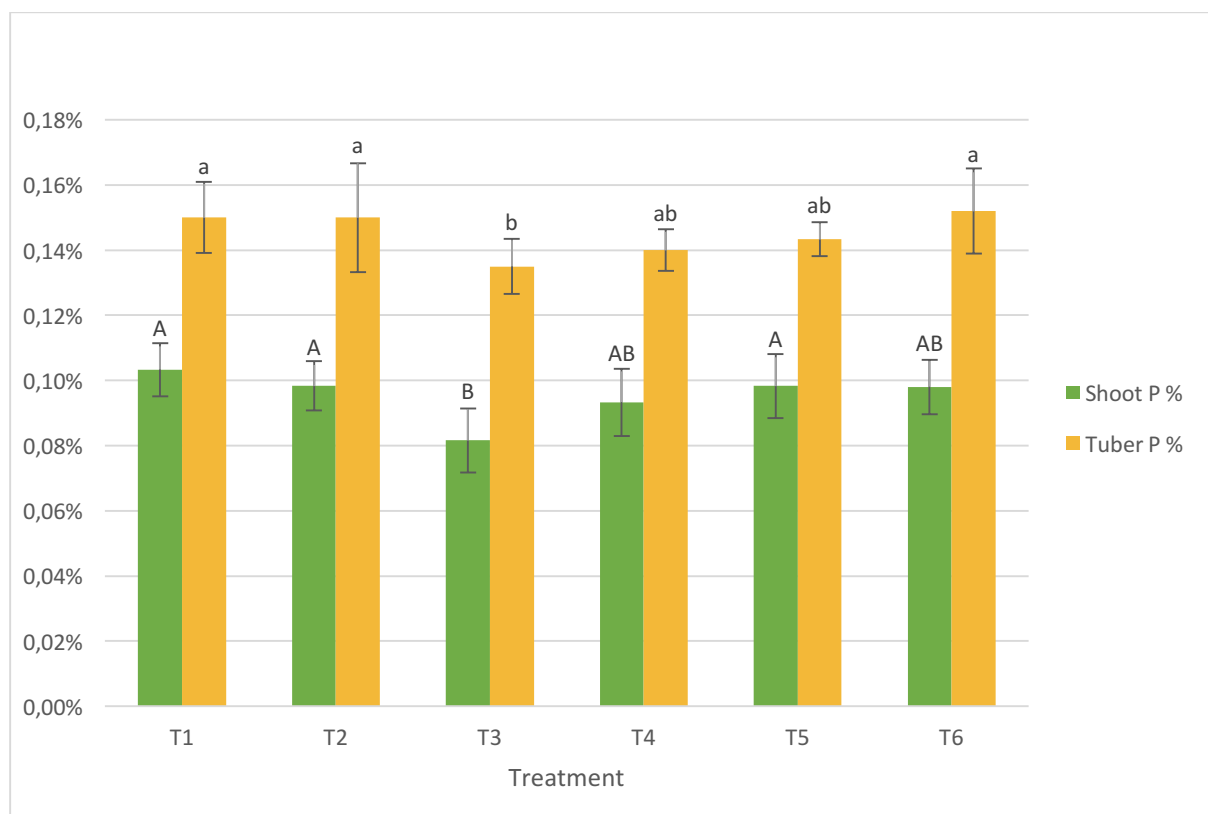


Figure 3: P concentration in shoots and tubers at harvest (60 dae) (DM). Mean values, $n=6$, \pm SD.

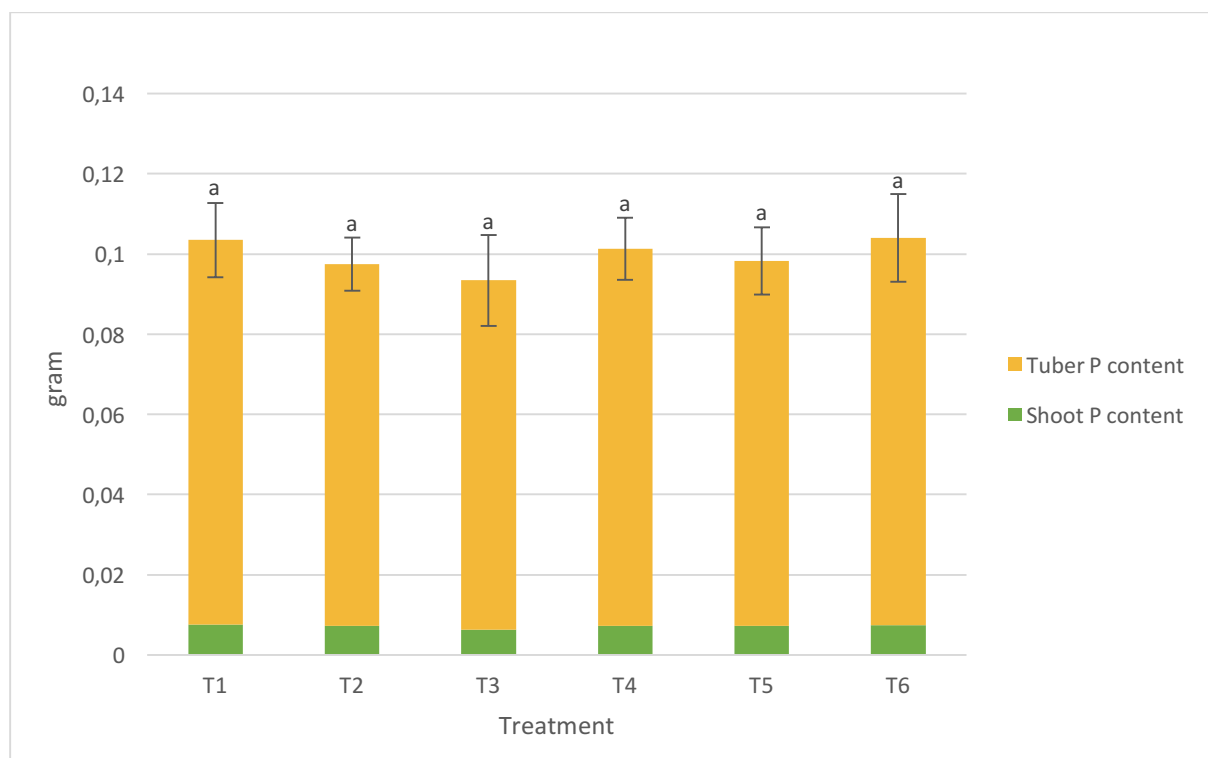


Figure 4: Total P content at harvest (60 dae). Mean values, $n=6$, \pm SD.

Mycorrhizal colonisation

AM colonization in the roots were found in all pots in the control T2 and the inoculated T5.

Discussion

Despite the high P sufficiency level indicated by both the P-AL and Olsen-P soil tests, none of the treatments affected the plant available P to such extent that the plants could access enough P for normal development. The growth was inhibited at an early stage which made it difficult to draw any conclusions about the effect of the different treatments. The reason was probably salt toxicity in combination with P deficiency.

P deficiency related to soil test results

According to Havlin (2005), the P-AL test (12.5 mg/100 g) indicates a high P sufficiency level, and the Olsen test (21 mg/1000 g) indicates a very high sufficiency level (table 7).

At this high P-AL levels the Swedish Board of Agriculture recommends application of just enough P to compensate for the P being removed by the crop (0.5 kg P/tonne harvested tubers) to avoid depleting the soil over time (Albertsson *et al.*, 2015). However, because a P-AL test may overestimate the plant available P in alkaline soils (Mattsson *et al.*, 2001; Albertsson *et al.*, 2015), the P fertilizer recommendation for soils with pH > 7 is to use the recommendation for the lower P-AL class. Accordingly, a potato field with an estimated yield of 30 tonnes per hectare, and this trial's soil, would need 30 kg P/hectare (table 9) (Albertsson *et al.*, 2015).

The Olsen-P test however, which is considered to give a more accurate indication of the amount of plant available P in alkaline soils, indicates a very high P sufficiency level, corresponding to P-AL class V (Havlin, 2005). The Olsen-P test is even reported to underestimate the amount of plant available P in soils with high Olsen-P levels (Horta and Torrent, 2007). Following the recommendations from the Swedish Board of Agriculture, only enough P to compensate for the P being removed by the crop, 15 kg P per hectare, needs then to be supplied. This means that the P in the soil should be enough to cover this season's crop.

Table 7: Gradation of P-values from P-AL test and Olsen-P test (adapted from Havlin, 2005). The Swedish Board of Agriculture's P-fertilizer recommendations for early potatoes based on P-AL and a yield of 30 tonnes/hectare, adjusted with 0.5 kg P per tonne deviation (Albertsson et al., 2015).

| Sufficiency level | P-AL mg/100 g | P-AL class | Olsen-P mg/1000 g | P-fertilizer recommendations kg/ha pH < 7 | P-fertilizer recommendations kg/ha pH > 7 |
|-------------------|------------------|---------------|-------------------|----------------------------------------------------|----------------------------------------------------|
| Very low | < 2 | I | < 3 | 70 | |
| Low | 2.0-4.0 | II | 4-7 | 50 | 70 |
| Medium | 4.1-8.0 | III | 8-11 | 40 | 50 |
| High | 8.1-16 | IV | 12-20 | | |
| High | 8.1-12 | IVA | | 30 | 40 |
| | 12.1-16 | IVB | | 15 | 30 |
| Very high | > 16 | V | > 20 | 15 | 15 |

In the present study, the first signs of P deficiency were evident 19 dae and within 36 dae all plants had symptoms which then accelerated during the cultivation period. At harvest the P concentrations in the plant tissues was considerably lower than the P concentration in a healthy plant. Walworth and Muniz (1993) reported that tubers which are sufficiently supplied with P contain at least 0.21 % P at 74 days after planting (dap). In the present study, the tuber P concentration 77 dap was only 0.14 – 0.15 %. The largest part of the P is taken up by the potato plant between 40 - 80 dae, and during this period the P uptake rate is at its highest (Kolbe and Stephan-Beckmann, 1997). The P deficiency symptoms emerged early, when the P demand by the crop was relatively low (Kolbe and Stephan-Beckmann, 1997). In the present experiment, the soil temperature was around 10 – 16 °C until 31 dae, which was considerably warmer than the mean field temperature during the same development stage of early potatoes (see table 5). Low temperature alone is therefore probably not enough to explain the deficiency, but the young potato plants' limited root systems might be a part of the explanation. Even though the plants did not show any signs of salt toxicity when the first P deficiency symptoms emerged, salinity might have played a role after the second fertilizer addition, as high salt concentrations in the soil solution have been shown to reduce phosphate uptake in crops grown in soil (Grattan and Grieve, 1999). If the possible influence of salt toxicity is not taken into account, it can be concluded that neither the P-AL test or Olsen-P test gave an accurate picture of the amount of plant available P. The Olsen-P test gave the most inaccurate indication, given that Havlin's gradation is correct (Havlin, 2005).

P deficiency related to pH

Considering the soil's humus- and clay content (3.1 % and 17 % respectively), the optimum pH for nutrient uptake by the plants would have been 6.3 (table 8) (Albertsson *et al.*, 2015).

Table 8: Target pH for soils of varying clay and humus content (adapted after Albertsson *et al.*, 2015).

| Humus content % | Clay content % | | | | | |
|--------------------|----------------|--------|---------|---------|---------|------|
| | < 5 | 5 - 15 | 15 - 25 | 25 - 40 | 40 - 60 | > 60 |
| < 6 | 6.0 | 6.2 | 6.3 | 6.4 | 6.5 | 6.5 |
| 6 - 12 | 5.8 | 5.9 | 6.0 | 6.1 | 6.2 | 6.2 |
| 12 - 20 | 5.5 | 5.6 | 5.7 | 5.8 | 5.9 | 5.9 |
| 20 - 40 | 5.2 | 5.3 | 5.4 | 5.5 | 5.6 | 5.6 |
| > 40 | 5.0 | 5.1 | 5.2 | 5.3 | 5.4 | 5.4 |

The results from the present study indicate that none of the treatments were able to lower the rhizosphere pH enough, at least not at the right time. Apart from the nitrification process, the acidifying effect of the NH_4^+ ion is a result of active root uptake. In the beginning of the cultivation period, the uptake rate is slow due to the smaller plants, but as the plant grows the uptake rate accelerates to reach a peak about 45 dae (Kolbe and Stephan-Beckmann, 1997). It seems reasonable to assume that at 19 dae, when the first P deficiency symptoms were observed, too little NH_4^+ had been taken up by the roots to cause a pH reduction big enough to influence the plant available P in the soil solution in a significant way. This conclusion is reinforced by the fact that at 19 dae there were no differences in P deficiency symptoms between the NH_4 and NO_3 treatments.

The effect of ammonium sulphate versus ammonium chloride

Table 9: The different N treatments compared

| Treatment | | Treatment | | Result |
|-----------|-------------------------------------------------|-----------|-------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| T1 | (NH ₄) ₂ SO ₄ | T2 | NH ₄ NO ₃ | No significant differences between treatments. |
| T1 | (NH ₄) ₂ SO ₄ | T3 | NH ₄ Cl | T3 has a significant lower plant P concentration than T1. No significant differences in dry weight. |
| T1 | (NH ₄) ₂ SO ₄ | T4 | KNO ₃ Ca(NO ₃) ₂ | No significant differences between treatments. |
| T2 | NH ₄ NO ₃ | T3 | NH ₄ Cl | T3 has a significant lower plant P concentration than T2. No significant differences in dry weight. |

Table 9 shows a comparison of the results of the different N treatments. The significantly lower plant P concentration in the NH₄Cl treatment compared to the (NH₄)₂SO₄ treatment and the lack of difference between the treatments in dry weight indicates that the SO₄⁻ ion might be important for the effect of (NH₄)₂SO₄ on P uptake. Alternatively, the Cl⁻ ion in NH₄Cl is affecting the P uptake negatively. The difference might be explained by differences in the acidifying effect between the treatments. Both NH₄Cl and (NH₄)₂SO₄ have an acidifying effect in soil, but the acidifying effect of (NH₄)₂SO₄ has in long term field trials been shown to be larger than the effect of NH₄Cl when equal amounts of NH₄-N was applied (Khonje *et al.*, 1989). However, if the difference could be explained only by a pH effect, there should have been an even larger difference between the (NH₄)₂SO₄ treatment and the NH₄NO₃ and KNO₃ + Ca(NO₃)₂ treatment. According to Albertsson *et al.* (2015), (NH₄)₂SO₄ has an acidifying effect corresponding to a lime requirement of 3 kg CaO/kg N, whereas the need for liming when NH₄NO₃ is used as fertilizer is 1 kg CaO/kg N. KNO₃ and Ca(NO₃)₂ on the other hand reduce the need of liming (Albertsson *et al.*, 2015). As there were no differences in P concentration or dry matter between those treatments, this indicates that the lower P concentration in the NH₄Cl treatment cannot be explained by a pH effect only. The NH₄Cl treatment does not only differ significantly in P concentration compared to the (NH₄)₂SO₄ treatment, but also compared to NH₄NO₃. According to Khonje *et al.*, (1989), the acidifying effect of NH₄Cl is larger than the effect of NH₄NO₃. This points to the conclusion that the difference in P uptake between the NH₄Cl treatment and the (NH₄)₂SO₄ and NH₄NO₃

treatment respectively, presumably can be attributed to a negative effect caused by the Cl^- content compared to the SO_4^{2-} content and not to a positive effect on P uptake caused by the more acidifying effect of $(\text{NH}_4)_2\text{SO}_4$.

Salt toxicity due to high Cl^- concentrations in the NH_4Cl treatment might explain the significantly lower plant P concentration in the NH_4Cl treatment compared to the $(\text{NH}_4)_2\text{SO}_4$ treatment. In a review on the effects of salinity on mineral nutrition in horticultural crops, Grattan and Grieve (1999) conclude that salinity in general reduces phosphate uptake in crops grown in soil. Furthermore, the review concludes that crops are generally more tolerant to sulphate-salinity than to chloride-salinity. It has been suggested that for most vegetable crops the salt tolerance is 2 dS/m greater for sulphate compared to chloride (Grattan and Grieve, 1999). In a hydroponically conducted experiment on potato, both Cl^- and SO_4^{2-} were found to impair the uptake of phosphate by the root to the same extent (Hang, 1993). What differentiated the ions was their inhibition of the long distance transport of P to the shoots, where the inhibition by Cl^- was greater than the inhibition by SO_4^{2-} .

The amount of chloride applied to the pots corresponded to a dose of 520 kg Cl/hectare or 260 mg Cl/kg dry soil, if calculated on the basis of the pot surface' share of a hectare. Potato is considered to be sensitive to Cl (Pais and Benton Jones Jr, 1997). In trials in the 60's in the United States, where the effect of KCl was compared to KSO_4 in potato, a lower uptake of P was observed under KCl fertilization (Berger *et al.*, 1961). However, a review by Xu *et al.* (1999) on chlorides behaviour in the soil environment in agricultural systems, highlights the idea that there exists an optimal or critical level of Cl for maximal P uptake that varies between crops. Wang *et al.* (1989) observed that 300 - 450 mg Cl /kg soil was an optimal level for maximal P uptake. If Cl concentration was below this level, application of Cl stimulated P uptake, and if Cl concentration exceeded this level, P uptake was suppressed by additional Cl application. Xu *et al.* stated that in general, 500 mg Cl/kg soil, seemed to be a toxicity threshold for potato grown in soil (Xu *et al.*, 1999). Soil type is likely to influence the effect of Cl salinity. The negative effects of high Cl concentrations have been reported to be greater on light soils compared to clay soils (Van Loon and Van den Berg, 2003). A possible explanation would be lower concentrations of Cl in the soil solution in a clay soil due to its higher soil water content. According to this reasoning, the soil in this trial with a water content of 23 % at full field capacity, should have had a relatively high toxicity threshold for Cl^- .

Taking these numbers into account, 260 mg Cl/kg soil is unlikely to alone explain the lower P uptake under the NH_4Cl treatment compared to the $(\text{NH}_4)_2\text{SO}_4$ and NH_4NO_3 treatments. As all plants in all treatments showed symptoms of salt stress, and all plants had low concentrations of P in shoots and tubers indicating P deficiency, it seems more likely that the Cl content acted as “the straw that breaks the camel's back” in an already stressful root environment.

The two nutrient ions that were unbalanced between the treatments were SO_4^{2-} and Cl^- , which resulted in different salt concentrations and thereby different osmotic potentials in the soil solution. The NH_4Cl treatment contained 50 mmol SO_4^{2-} and 90 mmol Cl^- per pot, which in total were 140 mmol per pot. The $(\text{NH}_4)_2\text{SO}_4$ treatment contained 95 mmol SO_4^{2-} per pot and no Cl^- , and the NH_4NO_3 treatment contained only 50 mmol SO_4^{2-} and no Cl^- . The difference in total salt concentration is likely to account for the differences in P uptake, especially as this difference is reinforced by the fact that crops in general are more sensitive to chloride-salinity than to sulphate-salinity. This conclusion is supported by Grattan and Grieve's (1999) statement that salinity in general reduces phosphate uptake in crops grown in soil.

The effect of *Bacillus megaterium* and mycorrhizal fungi

Bacillus megaterium

As shown in table 10, there were no significant differences between the pots inoculated with *Bacillus megaterium* and the control in terms of tuber yield, P uptake or fresh or dry matter. However, a significant correlation was found between water consumption and dry matter, and even though no significant difference was found in water consumption between the inoculated plants and the control, the Fischer LSD test did show a difference between the two treatments (table 6). Even though it is not possible to draw any conclusions from this, it would have been interesting to see if the trend had been reinforced if the plants had been allowed to reach full maturity, as other trials have shown that inoculation with *Bacillus* spp. in potatoes might enhance plant growth and tuber yield (Ekin *et al.*, 2009; Hosni *et al.*, 2016).

Mycorrhizal fungi

The finding of AM colonization in both the inoculated treatment and the control treatment contradicted the findings of a Swedish study by Ohlsson *et al.* (2011) where no AM colonization was found in potato grown in field in soil with medium to very high sufficiency

levels of P. The absence of AM colonization was explained by early sampling, a supposed inhibitory effect caused by high soil P levels, and a possible negative effect caused by fungicides. The soil in this trial had similar characteristics; a high P sufficiency level and many years of exposure to fungicides. What differed was the time of sampling, where Ohlsson *et al.* (2011) sampled the roots 20 - 25 dae, compared to 60 dae in this trial. The longer growth period can probably alone explain why AM colonization was possible to detect in this trial's potato roots and not in Ohlsson *et al.*'s study. The result is in accordance with the findings in Hijri's meta study (2016), where AM colonization of potato roots was found in all conventional potato fields, both inoculated with AM spores and non-inoculated.

Table 10: The inoculated treatments compared to the control

| Treatment | | Treatment | | Result |
|-----------|---------------------------------|-----------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| T2 | NH ₄ NO ₃ | T5 | NH ₄ NO ₃ Mycorrhizal inoculum ¹ | No significant differences between treatments. |
| T2 | NH ₄ NO ₃ | T6 | NH ₄ NO ₃ <i>Bacillus</i> <i>megaterium</i> ² | Tukey test: No significant differences between treatments. Fischer LSD test: T6 consumed significantly more water than T2 (table 6). Pearson correlation: Dry matter and water consumption significantly correlated (p < 0.001) |

1) *Rhizophagus irregularis*, inoculum produced by Symplanta, 10 000 spores/gram.

2) Product name P SOL B, produced by AgriLife, www.agrilife.in, 1.0 * 10⁹ CFU/g.

Conclusions

The aim of this study was to assess the possibility to control P availability for early potatoes, grown in alkaline soils with relatively high P content, through the choice of nitrogen source or through inoculation with PSB or AMF. The results, however, did not support the hypothesis that using ammonium sulphate as nitrogen source would result in higher P uptake by plants than using ammonium nitrate, ammonium chloride or potassium nitrate as nitrogen sources. Nor did the results support the hypothesis that inoculation with the PSB *Bacillus megaterium* or spores of the AMF *Rhizophagus irregularis* would result in higher P uptake by plants compared to the uninoculated control. One possible reason why no differences were found between treatments was that the plants probably were exposed to salt stress which hampered their development. It would therefore be interesting to redo the experiment with lower doses of nutrients to see the results of the different treatments on more developed plants, where P deficiency would be the only limiting factor.

References

- Agrilife, 2016. P Sol B product information. Homepage (online): http://www.agrilife.in/bioferti_psolb_bm.htm (accessed 2016-06-10).
- Albertsson, B., Börling, K., Kudsk, T., Kvarnmo, P., 2015. *Riktlinjer för gödsling och kalkning 2015*. Report JO14:12, Swedish Board of Agriculture.
- Bennett, W.F., 1993. Nutrient deficiencies & toxicities in crop plants. The American Phytopathological Society.
- Berger, K.C., Potterton, F.E., Hobson, E.L., 1961. *Yield, quality, and phosphorus uptake of potatoes as influenced by placement and composition of potassium fertilizers*. American Potato Journal. 38, 272–285. doi:10.1007/BF02862172
- Cao, W., Tibbitts, T.W., 1993. *Study of various NH_4^+ / NO_3^- mixtures for enhancing growth of potatoes*. Journal of Plant Nutrition, 16, 1691–1704. doi:10.1080/01904169309364643
- Douds, D.D., Nagahashi, G., Reider, C., Hepperly, P.R., 2007. *Inoculation with arbuscular mycorrhizal fungi increases the yield of potatoes in a high P soil*. Biological Agriculture and Horticulture, 25, 67–78. doi:10.1080/01448765.2007.10823209
- Duffy, E.M., Cassells, A.C., 2000. *The effect of inoculation of potato (Solanum tuberosum L.) microplants with arbuscular mycorrhizal fungi on tuber yield and tuber size distribution*. Applied Soil Ecology 15, 137–144.
- Ekin, Z., Oguz, F., Erman, M., Ögün, E., 2009. *The effect of Bacillus sp. OSU-142 inoculation at various levels of nitrogen fertilization on growth, tuber distribution and yield of potato (Solanum tuberosum L.)*. African Journal of Biotechnology, 8, 4418–4424. Available online at <http://www.ajol.info/index.php/ajb/article/viewFile/62394/50365>.
- Eriksson, J., 2011. Marklära, 1. uppl. ed. Studentlitteratur, Lund.
- Faccini, G., Garzón, S., Martínez, M., Varela, A., 2007. *Evaluation of the effect of a dual inoculum of phosphate-solubilizing bacteria and Azotobacter chroococcum, in crops of creole potato (papa “criolla”), “yema de huevo” variety (Solanum phureja)*. First International Meeting on Microbial Phosphate Solubilization, Developments in Plant and Soil Sciences, 102, 301–308.
- Fan, M.S., Li, Z., Wang, F.M., Mang, J.H., 2009. *Growth and phosphorus uptake of oat (Avena nuda L.) as affected by mineral nitrogen forms supplied in hydroponics and soil culture*. Pedosphere, 19, 323–330.
- Gigon, A., Rorison, I.H., 1972. *The Response of some ecologically distinct plant species to nitrate- and to ammonium-nitrogen*. Journal of Ecology, 60, 93–102. Available online at <http://www.jstor.org/stable/2258043>.
- Grattan, S.R., Grieve, C., 1999. *Salinity-mineral nutrient relations in horticultural crops*. Scientia Horticulturae, 78, 127–157. Available online at [http://dx.doi.org/10.1016/S0304-4238\(98\)00192-7](http://dx.doi.org/10.1016/S0304-4238(98)00192-7).
- Hagman, J., 2011. *Resultat från sommarens färskpotatisförsök*. Department of Crop Production Ecology, Swedish University of Agricultural Sciences. Available online at [http://www.skaneforsoken.nu/dokument/L7-710\(1\).pdf](http://www.skaneforsoken.nu/dokument/L7-710(1).pdf)
- Hang, Z., 1993. *Influence of chloride on the uptake and trans-location of phosphorus in potato*. Journal of Plant Nutrition, 16, 1733–1737. doi:10.1080/01904169309364646
- Havlin, J., 2005. Soil fertility and fertilizers: an introduction to nutrient management, 7th edition. Pearson Prentice Hall, Upper Saddle River, NJ.
- Hayman, D.S., 2011. *The physiology of vesicular–arbuscular endomycorrhizal symbiosis*. Canadian Journal of Botany, 61, 944–963 doi:10.1139/b83-105.
- Hijri, M., 2016. *Analysis of a large dataset of mycorrhiza inoculation field trials on potato shows highly significant increases in yield*. Mycorrhiza, 26, 209–214. doi:10.1007/s00572-015-0661-4.
- Hinsinger, P., 2001. *Bioavailability of soil inorganic P in the rhizosphere as affected by root-*

- induced chemical changes: a review*. Plant and soil, 237, 173–195.
- Horta, M. do C., Torrent, J., 2007. *The Olsen P method as an agronomic and environmental test for predicting phosphate release from acid soils*. Nutrient Cycling in Agroecosystems, 77, 283–292. doi:10.1007/s10705-006-9066-2.
- Hosni, F., Asgharzadih, A., Ardakani, M., Hamidi, A., 2016. *The impact of potato mini-tuber inoculation with plant growth promoting rhizobacteria on tuber yield and nutrients uptake*. Crops Improvement, 17, 911–924.
- Johnson, C.N., Fisher, P.R., Huang, J., Vetanovetz, R.P., Argo, W.R., 2010. *Quantifying the acidity of an ammonium-based fertilizer in containerized plant production*. HortScience 45, 1099–1105.
- Khonje, D.J., Varsa, E.C., Klubek, B., 1989. *The acidulation effects of nitrogenous fertilizers on selected chemical and microbiological properties of soil*. Communications in Soil Science and Plant Analysis, 20, 1377–1395. doi:10.1080/00103628909368156.
- Kolbe, H., Stephan-Beckmann, S., 1997. *Development, growth and chemical composition of the potato crop (Solanum tuberosum L.). II. Tuber and whole plant*. Potato Research, 40, 135–153.
- Kucey, R. M., 1983. *Phosphate-solubilizing bacteria and fungi in various cultivated and virgin Alberta soils*. Canadian Journal of Soil Science, 63, 671–678.
- Lindsay, W.L., 1979. *Chemical equilibria in soils*. John Wiley and Sons Ltd., Chichester, Sussex, UK.
- Malboobi, M.A., Behbahani, M., Madani, H., Owlia, P., Deljou, A., Yakhchali, B., Moradi, M., Hassanabadi, H., 2009. *Performance evaluation of potent phosphate solubilizing bacteria in potato rhizosphere*. World Journal of Microbiology and Biotechnology, 25, 1479–1484. doi:10.1007/s11274-009-0038-y.
- Marschner, H., 1995. Mineral nutrition of higher plants, 2nd ed. Academic Press, London.
- Marschner, H., Römheld, V., Cakmak, I., 1987. *Root-induced changes of nutrient availability in the rhizosphere*. Journal of Plant Nutrition, 10, 1175–1184.
- Mårtensson, A.M., Carlgren, K., 1994. *Impact of phosphorus fertilization on VAM diaspores in two Swedish long-term field experiment*. Agriculture, Ecosystems & Environment, 47, 327–334. doi:10.1016/0167-8809(94)90099-X.
- Mattsson, L., Börjesson, T., Kjell, I., Kjell, G., 2001. *Utvidgad tolkning av P-AL för mark-och skördeanpassad fosforgödsling*. Report 202. Swedish University of Agricultural Sciences, Dept. of Soil Sciences, Uppsala.
- McArthur, D.A., Knowles, N.R., 1993. *Influence of species of vesicular-arbuscular mycorrhizal fungi and phosphorus nutrition on growth, development, and mineral nutrition of potato (Solanum tuberosum L.)*. Plant Physiology 102, 771–782.
- Naderi, D.M., Nia, H.A., Bahari, B., 2012. *Assessment of applications of biological fertilizer for potato cultivation*. International Journal of AgriScience 2, 102–107.
- Gunnarsson, O., 1987. *Long-term dynamics of phosphorus in soils illustrated by means of soil analyses from long term fertility experiments*. Lectures and questionings at the conference held by The Royal Swedish Academy of Agriculture and Forestry at Arlöv, May 27th 1987.
- Ohlsson, O., Caspersen, S., Rådborg, T., Ekelöf, J., 2011. *Inventering av mykorrhiza i potatisförsök*. LTJ- faculty factsheet 2011:40, Swedish University of Agricultural Sciences, Alnarp.
- Pais, I., Benton Jones Jr, J., 1997. The Handbook of Trace Elements. CRC Press.
- Redecker, D., Kodner, R., Graham, L.E., 2000. *Glomalean fungi from the Ordovician*. Science, 289, 1920–1921. doi:10.1126/science.289.5486.1920.
- Richardson, A.E., 2001. *Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants*. Functional Plant Biology 28, 897–906.
- Sims, J.T., Sharpley, A.N., 2007. Phosphorus: agriculture and the environment, 2nd printing.

- American Society of Agronomy, Madison, Wis.
- SMHI, 2016. SMHI Open Data. Homepage (online): <http://opendata-download-metobs.smhi.se/explore/> (accessed 2016-10-03).
- Smith, S.E., Read, D.J., 2008. *Mycorrhizal Symbiosis*, 3rd edition. Elsevier Academic Press Inc, San Diego.
- Sylvia, D.M., 1998. *Principles and applications of soil microbiology*. Prentice Hall, Upper Saddle River, N.J.
- Taiz, L., Zeiger, E., Møller, I.M., Murphy, A.S., 2015. *Plant physiology and development*, 6th edition. Sinauer Associates, Inc.
- Van Loon, C.D., Van den Berg, W., 2003. *The effect of chloride fertilization on blackspot susceptibility and other quality characteristics and on yield of potato*. *Potato research*, 46, 147–154.
- Vessey, J.K., 2003. *Plant growth promoting rhizobacteria as biofertilizers*. *Plant Soil*, 255, 571–586. doi:10.1023/A:1026037216893.
- Vierheilig, H., Coughlan, A.P., Wyss, U., Piché, Y., 1998. *Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi*. *Applied and Environmental Microbiology*, 64, 5004.
- Vosátka, M., Gryndler, M., 2000. *Response of micropropagated potatoes transplanted to peat media to post-vitro inoculation with arbuscular mycorrhizal fungi and soil bacteria*. *Applied Soil Ecology* 15, 145–152. doi:10.1016/S0929-1393(00)00090-1.
- Walworth, J.L., Muniz, J.E., 1993. *A compendium of tissue nutrient concentrations for field-grown potatoes*. *American potato journal* 70, 579–597.
- Yao, M., Tweddell, R., Désilets, H., 2002. *Effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated potato plantlets and on the extent of disease caused by Rhizoctonia solani*. *Mycorrhiza* 12, 235–242. doi:10.1007/s00572-002-0176-7.
- Wu, F., Wang, W., Ma, Y., Liu, Y., Ma, X., An, L., Feng, H., 2013. *Prospect of beneficial microorganisms applied in potato cultivation for sustainable agriculture*. *African Journal of Microbiology Research*, 7, 2150–2158.
- Xu, G., Magen, H., Tarchitzky, J., Kafkafi, U., 1999. *Advances in Chloride Nutrition of Plants*, in: *Advances in Agronomy*. Elsevier, 97–150.
- Yao, M., Tweddell, R., Désilets, H., 2002. *Effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated potato plantlets and on the extent of disease caused by Rhizoctonia solani*. *Mycorrhiza* 12, 235–242. doi:10.1007/s00572-002-0176-7.
- Yara, 2016a. Gödslingsråd för potatis. Homepage (online) (Swedish): http://www.yara.se/vaxtnaring/grodor/potatis/godsling-av-potatis/programs/SE_potato-crop-program (accessed 2016-08-21).
- Yara, 2016b. NPK 11-5-18 product information. Homepage (online) (Swedish): https://c4produktkatalog.lantmannen.se/index.php/component/virtuemart/?page=shop.product_details&flypage=flypage.tpl_mol&product_id=8483&category_id=9805 (accessed 2016-08-21).
- Zhang, F., Kang, S., Zhang, J., Zhang, R., Li, F., 2004. *Nitrogen fertilization on uptake of soil inorganic phosphorus fractions in the wheat root zone*. *Soil Science Society of America Journal*, 68, 1890–1895.